



Scholars Research Library

Der Pharma Chemica, 2010, 2(4): 409-416

(<http://derpharmachemica.com/archive.html>)



A simple and sensitive ion chromatography method for the determination of Ethanolamine in pharmaceutical drug substances

S. John Prasanna*^a, K. S. R. Pavan Kumar^a, K. Mukkanti^b, Vundavilli Jagadeesh Kumar^a
and Hemant Kumar Sharma^a

^aAurobindo Pharma Ltd. Research Centre, Hyderabad, Andhra Pradesh, India

^bJ. N. T. University, Hyderabad, Andhra Pradesh, India

ABSTRACT

A simple and sensitive ion chromatography method was developed and optimized for the determination of content of ethanolamine at very low levels in pantoprazole sodium, amlodipine besylate and carvedilol pharmaceutical drug substances. The mobile phase was consisted of 4mmole of tartaric acid and 1mmole of dipicolinic acid in water using METROSEP C 2 250 (Metrohm, 250mm x 4.0mm, 7 μ m particle size) column at ambient temperature. The retention time of ethanolamine was about 10 min and the total acquisition time was 25 min. The optimized method was validated to prove its performance characteristics by demonstrating selectivity, sensitivity (limit of detection and quantification), linearity, precision and accuracy. The established limit of detection and limit of quantification of ethanolamine was found to be 67ng/ml and 204ng/ml respectively.

Key words: Ion chromatography, ethanolamine, pantoprazole sodium, carvedilol, amlodipine besylate, validation.

INTRODUCTION

Ethanolamine (2-Aminoethanol) is widely used in the pharmaceutical industry, in applications such as synthesis of chemical intermediate for drug substances, stabilizer and controlling of pH in the reaction medium. Ethanolamine is irritating to the skin, eyes and lungs and at high concentrations; it may cause central nervous system depression in exposed to animals. The exposure guidelines for ethanolamine allow a threshold limit value (TLV) of 3ppm the LD(50) for acute oral exposure are 2,140 mg/kg and 700 mg/kg in rats and mice respectively. The dermal LD(50) in rabbits is 1000 mg/kg [1]. It was noted that, any impurity other than active moiety is

to be controlled with suitable limits in the drug substance irrespective of its harmful nature as per International Conference on Harmonization (ICH) guidelines on impurities [2]. However, the analysis of ethanolamine presents numerous distinctive challenges due to its low molecular weight (61.0833 amu), having high polarity and relatively low volatility, basic in nature, easily ionizable in aqueous media and lack of UV absorbing chromophore. A lack of chromophore in ethanolamine, it is difficult to determine convenient HPLC method using UV photometric detection method. Various techniques were employed for the determination of ethanolamine in different types of samples in literature like, in amniotic fluid by capillary electrophoresis method with contactless conductivity detection mode [3], in Spanish wines by using high performance liquid magneto-chromatography [4], in ground water samples by using cation exchange chromatography with suppressed conductivity detection [5] and also, in air samples by ion chromatography [6], in cheese, by using ultra performance liquid chromatography(UPLC)[7], in drug substances by derivatization with Marfey's reagent by HPLC[8], determination of alkanolamines by ion-pair HPLC (High performance liquid chromatography) using a chemiluminescence detection [9]. Determination of trace amino alcohols in water, air and Bitumen-salt masses forming in the detoxication of chemical warfare agents by using gas chromatography [10] and determination of ethanolamine with phenyl isothiocyanate as derivatization agent by using HPLC method in musts and in wines [11] were also reported in literature. Various methods and modes of detection have been successfully applied for screening the ethanolamine in pharmaceuticals and in environmental samples. Ethanolamine exhibits as amphoteric properties; it is difficult to select a direct GC (Gas chromatography) analysis. Therefore, it requires a simple alternative analytical technique to evaluate the ethanolamine.

In present studies, a non-suppressor mode ion chromatography method was developed and optimized to determine the content of ethanolamine in Carvedilol, Amlodipine besylate and Pantoprazole sodium pharmaceutical drug substances. In Pantoprazole sodium drug substance, ethanolamine was used as a stabilizer and for controlling of pH. While in the case of Carvedilol and Amlodipine besylate, ethanolamine was used in preparation of drug intermediate and also the drug substances have ethanolamine moiety. The structures of Carvedilol and Amlodipine are depicted in the Fig.1 and ethanolamine moiety is encircled.

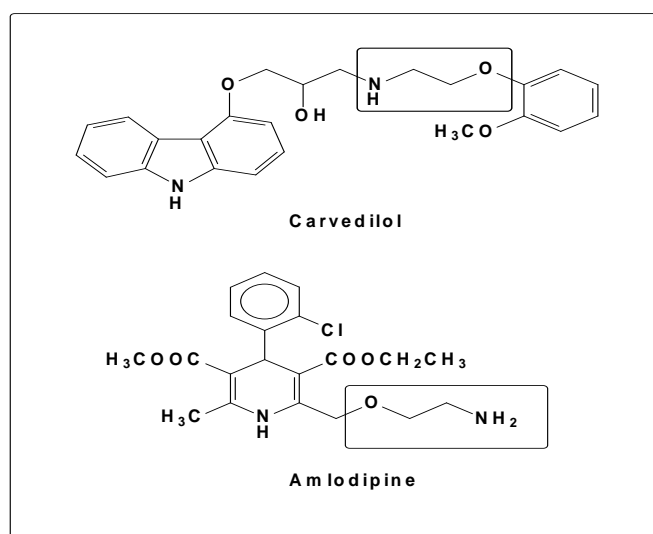


Fig. 1. The ethanolamine moiety in the structures of Carvedilol and Amlodipine

As Ion chromatography is a conventional analytical technique for the separation of inorganic anions, cations as well as low molecular weight organic acids and bases, ethanolamine, the analyte of interest exists as cation in aqueous solution in acidic medium. Selection of a suitable eluent, stationary phase and mode of detector influences the separation of ethanolamine from the drug substance matrix. The optimized IC method was validated according to ICH guidelines [12] to prove its suitability and reliability for the determination of ethanolamine in pharmaceutical drug substances during routine analysis.

MATERIALS AND METHODS

Chemicals, reagents and samples

Analytical-reagents grade of ethanolamine, lithium chloride, ammonium chloride, sodium chloride, potassium chloride, tartaric acid HPLC grade methanol and nitric acid were procured from E.Merck, India. Dipicolinic acid was purchased from Lancaster. Water was distilled and purified with Millipore system (Millipore Milford, MA USA). Pantoprazole sodium, carvedilol and amlodipine besylate pharmaceutical drug substances, related impurities were prepared from Aurobindo Pharma Ltd. Research Laboratories, Hyderabad, India.

Ion chromatography

An IC system, Metrohm 761 Compact IC consisting of conductometric detector and Metrohm 750 auto sampler was used. The data handling system was Metrohm 761 Compact IC software. The analysis was carried out on METROSEP C 2 250 (Metrohm, 250mm x 4.0mm, 7 μ m particle size) column packed with silica gel with carboxyl groups as stationary phase at ambient temperature. The mobile phase was delivered in an isocratic mode at a flow rate of 1.0 ml/min. The detector was operated in conductivity mode and analogue range of the detector was set at 1000 μ S/cm. The injection volume was 20 μ l. The retention time of ethanolamine was about 10 minutes. The total run time was 25 min.

Standard solution

Accurately weigh and transfer 100 mg of ethanolamine into a 50 ml volumetric flask, dilute to volume with water, dilute 5 ml of this solution to 50 ml with water and dilute 5 ml of this solution to 50 ml with water. Further dilute 5 ml of this solution to 100 ml with water. Filter the solution through 0.45 μ m or finer porosity membrane filter.

Sample solution

Preparation of pantoprazole sodium and amlodipine besylate sample solutions

Accurately weigh and transfer 100 mg of sample into a 100 ml volumetric flask, add 30 ml of water and dissolve by shaking and make up to the volume with water. Filter through the 0.45 μ porous membrane.

Preparation of carvedilol sample solution

Accurately weigh and transfer 100 mg of sample into a 100 ml volumetric flask, add 10 ml of methanol and dissolve by shaking and make up to the volume with 10mmole of nitric acid. Filter through the 0.45 μ porous membrane.

Preparation of mobile phase

Dissolve 600mg of tartaric acid and 167mg of dipicolinic acid in 500ml of water and make up to 1000ml with water. Filter through the 0.45 μ porous membrane.

RESULTS AND DISCUSSION**Method development**

Ethanolamine is a process impurity in various pharmaceutical drug substances. Most of the low molecular weight amines and inorganic cations which have no UV-Visible characteristics which limits detection options to amperometry, conductometry, refractive index or indirect methods. In ion chromatography, the low molecular weight organic bases have a greater affinity and easily bind/exchange with column stationary phase, which helps to determine the ionisable impurities in the drug substances. The determination of low molecular mass amines by IC was typically achieved by using either silica or resin based cation exchange column. The use of poly (butadiene-maleic acid) coated silica column and a mobile phase of EDTA-nitric acid containing acetonitrile or methanol was reported in literature. We initiated this present work with poly (butadiene-maleic acid) coated silica column and a mobile phase of 4mmole of tartaric acid and 1mmole of dipicolinic acid (which acts as complexing agent for separation of the cations) avoiding usage of organic modifiers. The ethanolamine peak was well separated with alkali metal ion peaks. Ethanolamine was co-eluted with high levels of sodium ion present in the sample solution. To overcome this limitation, alternatively, a column of carboxylic group on silica was employed and the resolution between sodium peak and ethanolamine satisfactorily improved. Weakening and increasing the ionic strengths of mobile phase didn't significantly change the resolution between the sodium and ethanolamine peaks. Finally, the ion chromatography method was optimized to use of 4mmole of tartaric acid and 1mmole of dipicolinic acid as eluent with conductometric detection.

This optimized IC method to determine the content of ethanolamine in various pharmaceutical drug substances was validated according to ICH guidelines [12] to evaluate its performance characteristics.

Validation

The experiments that have been demonstrated during validation studies were selectivity, sensitivity by means of limit of detection and quantification, linearity, precision (system precision, method precision), stability of sample solution and accuracy, and the results obtained from the experiments were briefly summarized below.

Selectivity

The solution of blank, standard solution of ethanolamine, lithium, sodium, potassium, ammonium and pharmaceutical drug substances were prepared separately, and injected as per procedure to identify the retention time of components of sample matrix. The each drug substance sample solution was spiked with ethanolamine and also each drug substance sample solution was spiked with known related substances of each of pharmaceutical drug substances along with ethanolamine were prepared separately and injected as per procedure to confirm any co-elution of peaks due to sample matrix. The chromatograms obtained from the analyses shows that, the ethanolamine peak was well resolved from background noise and from that of blank, lithium, sodium, potassium, ammonium and other components of sample matrix. It is well

indicating that the selectivity of the method to determine the content of ethanolamine in pharmaceutical drug substances. The overlay chromatogram of ethanolamine standard solution (1 ppm), pantoprazole sodium, carvedilol and amlodipine besylate drug sample separately spiked with known related substances of each drug substance including ethanolamine and blank solution are shown in Fig 2.

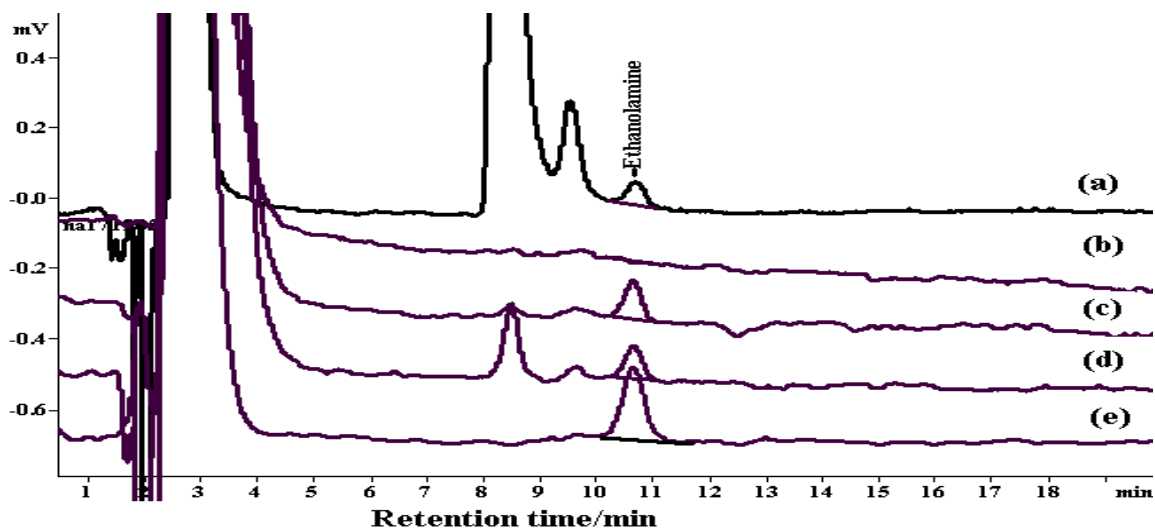


Fig. 2. a. Pantoprazole sodium drug substance spiked with all known impurities of pantoprazole sodium including Ethanolamine, b. Blank solution, c. Amlodipine besylate drug substance spiked with all its known impurities including Ethanolamine, d. Carvedilol drug substance spiked with all its known impurities including Ethanolamine, e. standard solution of Ethanolamine (1ppm)

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were predicted using slope (S) and residual standard deviation (SD) that obtained from a linear regression, is being one of the three approaches described in ICH guidelines [12]. The formula used for the prediction of LOD and LOQ were $3.3 \times SD/S$ and $10 \times SD/S$ respectively. The predicted LOD and LOQ levels were found to be 67ng/ml and 204ng/ml respectively. The solutions were prepared at the predicted concentration of LOD and LOQ levels, and analyzed for six times, and the percentage relative standard deviation was found to be 25.3 and 6.2 respectively. Thus, the LOD and LOQ values were established to determine the content of ethanolamine in pharmaceutical drug substances.

Linearity

The linear relationship of analyte of interest response against concentration was verified in the working concentration range by analyzing different level of solutions containing ethanolamine from about 0.1 μ g/ml to 1.28 μ g/ml. The linear regression line was plotted against analyte response versus concentration. The correlation coefficient of the regression line was found to be 0.999. The statistical analysis of linear regression line was evaluated and was summarized in Table 1.

Table 1: The statistical evaluation of linearity data

Statistical Parameter	Results
Correlation coefficient (r)	0.999
Concentration range ($\mu\text{g/ml}$)	Between 0.1 and 1.28
Intercept (a)	0.0565
Slope (b)	0.0039
Standard deviation of intercept	0.0302
Standard deviation of slope	2.21×10^{-4}
Standard error estimation	0.0797
Limit of detection (ng/ml)	67
Limit of quantification (ng/ml)	204

Precision (system precision and method precision)

System precision was demonstrated by analyzing six replicate injections of ethanolamine standard solution ($1\mu\text{g/ml}$) as per procedure. The percentage relative standard deviation of six replicate injections of ethanolamine standard solution performed on six different days was found to be less than 2.0. Repeatability of the test method (method precision) was demonstrated by analyzing six separate sample solutions were prepared using single batch of each pharmaceutical drug substance spiking with known amount of ethanolamine spiked in sample solution and the percentage relative standard deviation of ethanolamine content in six sample preparations were determined and it was found to be less than 5.0.

Stability of sample solution

The stability of sample solution at room temperature ($\approx 25^\circ\text{C}$) was evaluated by analyzing the sample solutions at different time intervals from initial to 14 hours. The percentage difference between the results obtained from initial and different time intervals was found to be less than 4.0, suggesting that the sample solution is stable for at least up to 14 hours at room temperature ($\approx 25^\circ\text{C}$) for determination of ethanolamine.

Accuracy

The accuracy of the method was verified by preparing sample solution spiked with known amount of ethanolamine at three different levels in the concentration range as described in the Table 2. Each concentration levels were prepared in triplicate and analyzed as per the method. The percentage recovery of the analyte was evaluated and it lies between 91 and 103 and the results are summarized in Table 2.

Table 2 Recovery results from spiking sample with ethanolamine

Spiked amount ($\mu\text{g/g}$)	Recovered amount ($\mu\text{g/g}$)	% Recovery	Statistical evaluation	
Pantoprazole sodium drug substance				
481	483	100.4		
479	464	96.9		
460	450	97.8		
971	969	99.8		
960	973	101.4		
942	922	97.9		
1152	1181	102.5	Over all Mean	100.1

1150	1187	103.2	SD	2.191
1185	1197	101.0	%RSD	2.2
Carvedilol				
488	497	101.8		
489	502	102.7		
492	493	100.2		
975	995	102.1		
977	978	100.1		
977	979	100.2		
1204	1105	91.8	Over all Mean	99.4
1174	1165	99.2	SD	3.435
1175	1129	96.1	%RSD	3.5
Amlodipine besylate				
487	496	101.8		
492	503	102.2		
489	491	100.4		
994	991	99.7		
989	977	98.8		
978	981	100.3		
1224	1241	101.4	Over all Mean	99.8
1251	1183	94.6	SD	2.277
1234	1223	99.1	%RSD	2.3

CONCLUSION

The level of ethanolamine in pharmaceutical drug substances is to be controlled / monitored during routine analysis to conform the desired purity of active moiety. This optimized ion chromatography is simple, uses of less reagents, shorter acquisition time, very sensitive and accurate. The results obtained from validation experiments proved that the ion chromatographic method used to determine the content of ethanolamine in pharmaceutical drug substances was selective, sensitive, linear, precise and accurate. Hence, this optimized ion chromatography method was suitable and reliable to determine the content of ethanolamine in pantoprazole sodium, amlodipine besylate and Carvedilol and may be useful for other pharmaceutical drug substances, where the ethanolamine is a possible contaminant in the drug substances.

Acknowledgements

The authors express their sincere gratitude to Aurobindo Pharma Ltd. Research Centre located in Hyderabad for providing the analytical support to pursue this work and we are also grateful to colleagues who helped us in this work.

REFERENCES

- [1] <http://www.osha.gov/SLTC/healthguidelines/ethanolamine/recognition.html>
- [2] International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline. Impurities in New Drug Substances Q3A(R2), step 4 **2006**.
- [3] P. Tuma, E. Samcova, K. Andelova, *J. Chromatogr. B.*, **1996**, 839, 12-18.
- [4] E. Barrado, J. A. Rodriguez, Y. Castrillejo, *Talanta.*, **2009**, 78, 672-675.
- [5] O. Mrklas, A. Chu, S. Lunn, *J. Enviro. Mon.*, **2003**, 5, 336-340.

- [6] A. Spiros, Bouyoucs, R. G. Melcher, *Am. Ind. Hyg. Asso. J.*, **1986**, 47(3), 185-188.
- [7] H. K. Mayer, G. Fiechter, E. Fischer, *J. Chromatogr. A.*, **2010**, 1217, 3251-3257.
- [8] K. K. Ngim, J. Zynger, B. Downey, *J. Chromatogr. Sci.*, **2007**, 45, 126-130.
- [9] P. J. Worsfold, *Anal. Chim. Acta.*, **1991**, 246, 447-450.
- [10] I. N. Stankov, A. A. Sergeeva, S. N. Tarasov, *J. Anal. Chem.*, **2000**, 55(2), 150-154.
- [11] M. Puig-Deu, S. Buxaderas, *J. Chromatogr. A.*, **1994**, 685, 21-30.
- [12] International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline, Validation of analytical procedures: Text and methodology Q2(R1), step 4 **2005**.
- [13] J. Krol, P. G. Alden, J. Morawski, *J. Chromatogr. .*, **1992**, 626, 165-170.